Further Evidences of Anomuran Non-pedunculate Spermatophores[1]

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MACRURAN NON-PEDUNCULATE SPERMATO-
PHORES were differentiated from anomuran
pedunculate spermatophores by Calman
(1909), but subsequent studies embracing
both macrura of the Palinuridae, Scyllaridae,
and Nephropsidae (Matthews, 1951, 1954*b*,
1954*c*) and the anomura of the Paguridae
(Mouchet, 1931; Matthews, 1953, 1956),
placed this differentiation in doubt. However,
not until it was discovered that a macruran,
Parribacus antarcticus, elaborated pedunculate
spermatophores (Matthews, 1954*a*) and an
anomuran, *Hippa pacifica*, elaborated non-
pedunculate spermatophores (Matthews,
1956) was the dichotomy between these two
tribes proven to be untenable.

This paper, which compares the develop-
ment of *Aniculus maximus* spermatophores
with that of certain other Paguridae, records
still other instances in which anomuran non-
pedunculate spermatophores are produced.

MATERIALS AND TECHNIQUES

Mature specimens of *Aniculus strigatus*
Herbst (Edmondson, 1946: 264; Forest, 1952:
19, *Trizopagurus strigatus* (Herbst)), collected
at Hanauma Bay, Oahu, T. H., during the
year 1954, and of *A. maximus* (*vide* Edmond-
son, *opus cit*. p. 79), obtained from the Hono-
lulu Aquarium during the year 1953, were
used in this study.

The right testis and vas deferens of each
specimen of *A. maximus* were fixed either in
Bouin's or Zenker's fluid, dehydrated and
cleared in dioxane, embedded in Tissuemat
(54–56°C.), and serially sectioned at 10 mi-
crons. The mounted sections were stained
either with Harris's alumhaematoxylin (coun-
terstained with eosin) or with Mallory's
Triple Stain.

The left vasa deferentia of both species
were immersed for 10–30 minutes in an aque-
ous solution of toluidine blue (1–10,000),
teased open in sea water, and their vitally
stained spermatophores studied.

OBSERVATIONS

In cross sections throughout the testis of
A. maximus immature sacculi containing pri-
mary spermatocytes, and more mature sacculi
containing secondary spermatocytes and sper-
matids, are observed. These sacculi, by the
renewed activity of their germinal epithelial
cells, produce new primary spermatocytes
which, as they fill the sacculi, force the meta-
morphosing spermatids into the minute col-
lecting tubule. Thus by combined, rhythmical
activities of numerous sacculi a continuous
sperm mass slowly enters the proximal por-
tion of the vas deferens where, molded in
compliance with the circular lumen, it forms
a rodlike sperm column.

Serial cross sections through this region of
the vas deferens disclose that the shape of the
lumen gradually changes from circular to
elliptical, synchronously effecting correspond-
ing changes in the shape of the sperm column.

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As the sperm column becomes elliptical, a secretion from the epithelial cells bounding the more pointed extremities of the elliptical lumen envelops the sperm column and forms the sperm column sheath.

Serial sections through somewhat more distal regions of the vas deferens disclose that the lumen gradually becomes pear-shaped. In longitudinal sections through this region (Fig. 1) the sheathed, sperm column appears as a series of partially closed arches (c), joined one to another by portions of the compressed, empty, sperm column sheath (d).

Longitudinal sections through slightly more distal regions of the vas deferens (Fig. 2) disclose that a secretion from the epithelial cells (f) bordering the narrow portion of the lumen forms the foot (e) and fills with precursory stalk material (d) the spaces between the closing arches.

Thus far, the histological and physiological phenomena of spermatophoric development in A. maximus parallel those of Dardanus punctulatus (Matthews, 1956) and Dardanus asper (Matthews, 1953: 260–262, figs. 7, 8, 9, 10, 11) in which truly pedunculate spermatophores are ultimately elaborated.

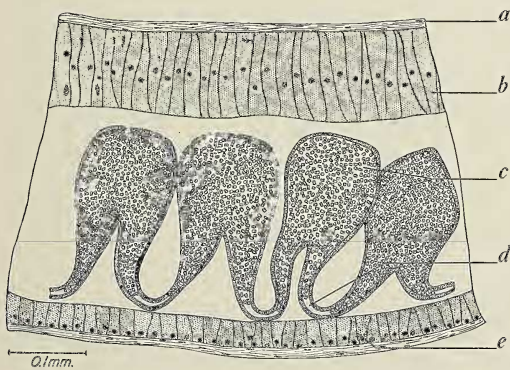In D. asper, as in A. maximus, the living vasa deferentia exhibit spasmodic contrac-

FIG. 1. Longitudinal section of the proximal vas deferens of Aniculus maximus showing: a, muscle layer; b, elongate epithelial cells; c, partially closed arches of sheathed, sperm column; d, compressed, empty sperm column sheath connecting partially closed arches; e, short, epithelial cells bounding narrow portion of lumen.

tions which serve both to move the sperm mass and to mold it in compliance with the gradually changing internal die, i.e., first cylindrical, then elliptical, then pear-shaped. In D. asper also, a secretion from epithelial cells isolated at opposite ends of the elliptical lumen forms the sperm column sheath. And this sheathed sperm column, by muscular contractions of the wall of the vas deferens, forms partially closed arches, joined one to another by portions of the compressed, empty, sperm column sheath. In D. asper, too, a secretion from the epithelial cells bordering the narrow portion of the pear-shaped lumen forms the foot and fills with precursory stalk material the spaces between the closing arches.

From this stage of development on, the processes in the elaboration of pedunculate and non-pedunculate spermotophores diverge. This divergence results, for the most part, from subsequent activities of the epithelial cells bounding the lumen.

In D. asper (op. cit., p. 263, figs. 12, 13), the epithelial cells at the narrow region of the pear-shaped lumen form a groove into which the precursor of the stalks is secreted. As the groove deepens, the stalk material lengthens both by the continued secretion of the epithelial cells and by the muscular contractions in the walls of the vas deferens. It is noteworthy that the lengthening of the stalks carries the ampullae of sperm "above" the foot and that the connecting sperm column sheaths between adjacent ampullae become extremely thin and finally obscure. Even in the short spermatophores of Birgus latro and Coenobita rugosus (Matthews, 1956) the stalks are lengthened sufficiently to carry the ampullae of sperm "above" the foot.

In A. maximus (Fig. 3) the epithelial cells (f) fail to form a deep groove into which the precursor of the stalks is secreted. Instead, this region of the lumen (d) widens and the secretion from the epithelial cells spreads out forming the broad foot (e). Noteworthy also is the sectioned portion of an arch (c), which here measures 112 microns high and 71 mi-
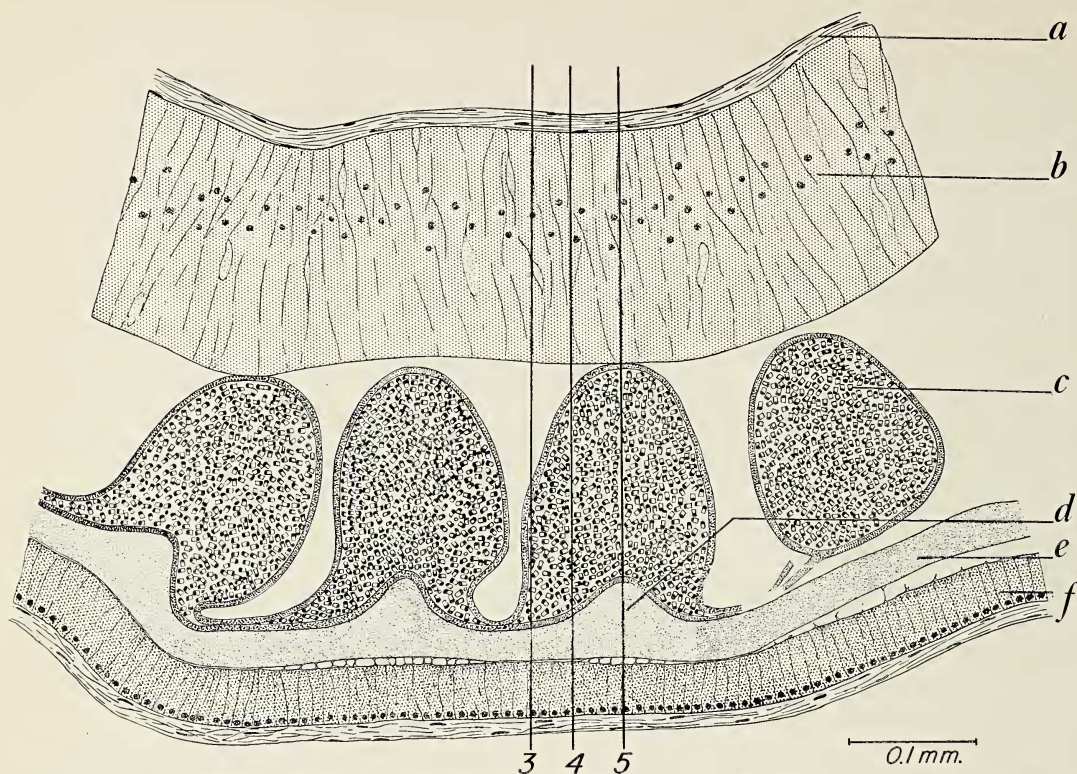
FIG. 2. Longitudinal section of the proximal vas deferens of *Aniculus maximus* (slightly distal to Fig. 1) showing: *a*, muscle layer; *b*, elongate epithelial cells; *c*, sectioned portion of arch; *d*, precursory stalk material; *e*, foot; *f*, short epithelial cells of narrow portion of lumen secreting the foot material. (The numbers 3, 4, 5 show regions through which Figs. 3, 4, 5 are taken.)

crons wide and appears like a distinct ampulla resting on the broad foot (*e*).

In subsequent sections through this region of the vas deferens (Fig. 4) although the foot forming epithelial cells (*f*) do not shorten, the epithelial cells (*b*) "above" the sectioned portion of the arch (*c*) attain a length of approximately 182 microns. Again it is noteworthy that the sectioned portion of the arch (*c*) appears like a distinct ampulla 171 microns high and 118 microns wide. Whereas, in Figure 3 the "ampulla" appeared to rest firmly on the broad, basal foot, in Figure 4 it is "elevated" approximately 12 microns by the sectioned portion of the stalk precursor (*d*).

In still other cross sections through this region of the vas deferens (Fig. 5) neither the foot forming epithelial cells (*f*) nor the epithelial cells (*b*) above the sectioned portion

of the arch (*c*) change appreciably in length. And again it is noteworthy that the sectioned portion of the arch (*c*) appears like a distinct ampulla, 171 microns high and 118 microns wide. The "ampulla" is elevated approximately 35 microns above the foot (*e*) by the sectioned portion of the stalk precursor (*d*).

The broad groove so characteristic of this portion of the lumen is no longer encountered in cross sections through the enlarged, apical portion of the vas deferens (Fig. 6). Gradually, the epithelium becomes folded and two typhlosole-like regions (*b*) appear at opposite sides of the lumen. From the epithelial cells bordering the crypts of these typhlosole-like folds, a new mucus-like secretion forms the matrix (*c*) which encompasses the completed spermatophores.

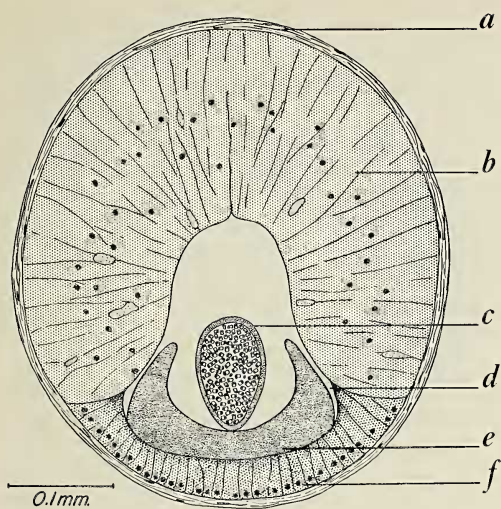Because of its tortuous nature, both cross sections (*e*) and longitudinal sections (*d*) of

FIG. 3. Cross section of the proximal vas deferens of *Aniculus maximus* (through region 3 of Fig. 2) showing: *a*, muscle layer; *b*, elongate epithelial cells; *c*, sectioned portion of arch; *d*, portion of wide lumen; *e*, foot; *f*, foot-forming epithelial cells.
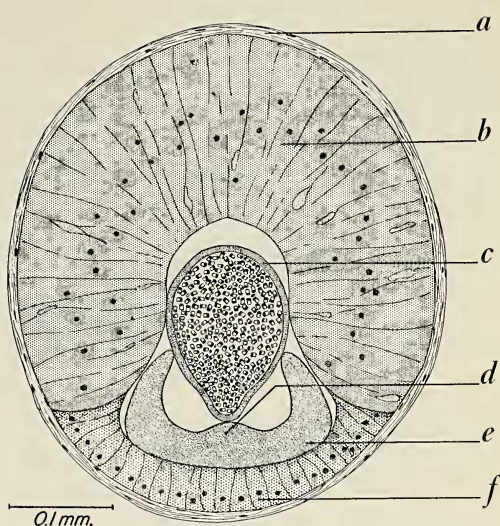

FIG. 4. Cross section of the proximal vas deferens of *Aniculus maximus* (through region 4 of Fig. 2) showing: *a*, muscle layer; *b*, elongate epithelial cells; *c*, sectioned portion of arch; *d*, stalk precursor; *e*, foot; *f*, foot-forming epithelial cells.

the spermatophore are here observed. Conspicuous also in these sections are the longitudinal muscles (*a*) which probably serve to eject the completed spermatophores (Fig. 7).

When the enlarged apical portions of the vasa deferentia of *A. strigatus* and *A. maximus* are placed in toluidine blue and their encompassing mucus-like matrices are dissolved (in 0.1N KOH), continuous, non-pedunculate spermatophores (Fig. 7) are revealed. Here is final evidence that the arches (*a*) never completely close forming distinct ampulla and that the arches are in reality never raised above the broad basal foot (*b*).

DISCUSSION

Mouchet (1931) assigns nine regions of activity to the vas deferens of the typical pedunculate spermatophore producing hermit crab, *Diogenes pugilator* Roux (Matthews, 1953: 264). The spermatophoric differences observed in *A. strigatus* and *A. maximus* are attributed to vasa deferentia which lack one or more of these regions. We are especially concerned in this discussion with region 4, in
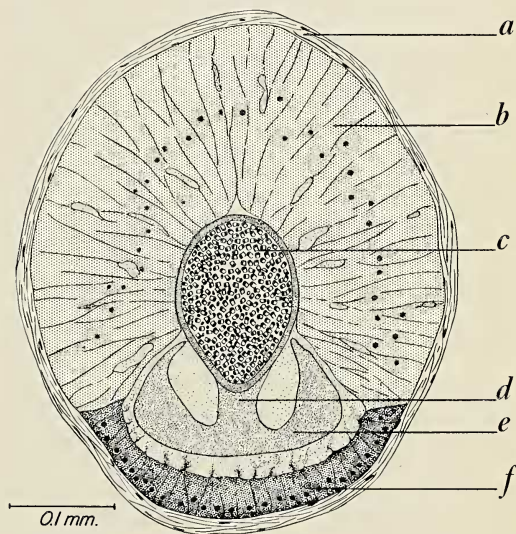

FIG. 5. Cross section of the proximal vas deferens of *Aniculus maximus* (through region 5 of Fig. 2) showing: *a*, muscle layer; *b*, elongate epithelial cells; *c*, sectioned portion of arch; *d*, stalk precursor; *e*, foot; *f*, foot-forming epithelial cells.

which each ampulla acquires a short thick stalk, and with region 6, in which the stalks are stretched.
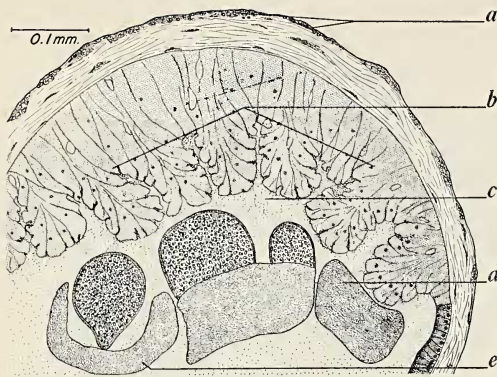
FIG. 6. Portion of cross section through enlarged distal vas deferens of *Aniculus maximus* showing: *a*, circular and longitudinal muscles; *b*, typhlosole-like region of epithelial cells; *c*, mucus-like matrix; *d*, longitudinal section through portion of spermatophore; *e*, cross section through portion of spermatophore.
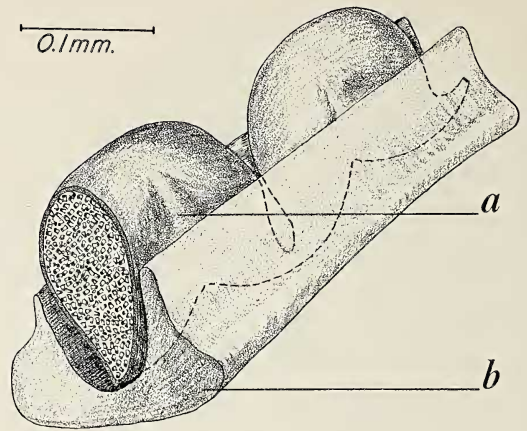

FIG. 7. A composite drawing of vitally stained extruded spermatophores of *Aniculus strigatus* or *Aniculus maximus* (with matrix dissolved away) showing: *a*, unclosed arch not elevated above foot; *b*, foot.

In my observation of the stained cross sections of *A. maximus* I am unable to determine with certainty whether region 4 is present or not. True, both cross and longitudinal sections reveal a "stalk precursor" which fills the spaces of the closing arches but this material, with the stains employed, could not, either in texture or staining affinity, be differentiated from that of foot. It is not the intent of this paper to base pedunculation on whether or not the "stalk precursor" is a separate and distinct secretion from that which forms the foot, but rather to point out that, despite its origin, region 6, where this secretion should be stretched, is lacking. The lack of this region has a pronounced effect on the ultimate elaboration of the spermatophores, for without it the arches are never truly "elevated above" the floor of the foot. That they may appear so in isolated cross sections of the vas deferens (Figs. 3, 4, 5) points to the need to consider both cross and longitudinal sections before statements concerning raised isolated ampullae can be made. Taken alone, Figure 3 and especially Figures 4 and 5 convey the erroneous impression that, indeed, the closed arches form isolated ampullae and that these are elevated on short stalks above the broad basal foot. In reality this is not true, as is seen when Figures 3, 4, and 5 are studied in relation to their position in the longitudinal section of the vas deferens (Fig. 2, regions 3, 4, 5).

This condition in *A. strigatus* and *A. maximus* is not new. Mouchet (1931) points out that the hermit crabs *Eupagurus bernhardus*, *E. prideauxi*, *E. cuanensis*, *Anapagurus hyndmanni*, and *Clibanarius misanthropus* likewise lack regions 4 and 6 in which the stalk is secreted and stretched. Although I have not investigated the spermatophoric development of these hermit crabs, it appears rather unlikely that distinct ampullae of sperm are elevated above the foot. If not, the spermatophores of these hermit crabs should be included with those of *A. strigatus* and *A. maximus* as further evidence of non-pedunculate spermatophore formation in the Paguridae.

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